

2012 Mixture Interpretation Workshop:

Mixtures Using *SOUND* Statistics, Interpretation, & Conclusions



Complexity Thresholds and Exclusion Criteria

Catherine M. Grgicak

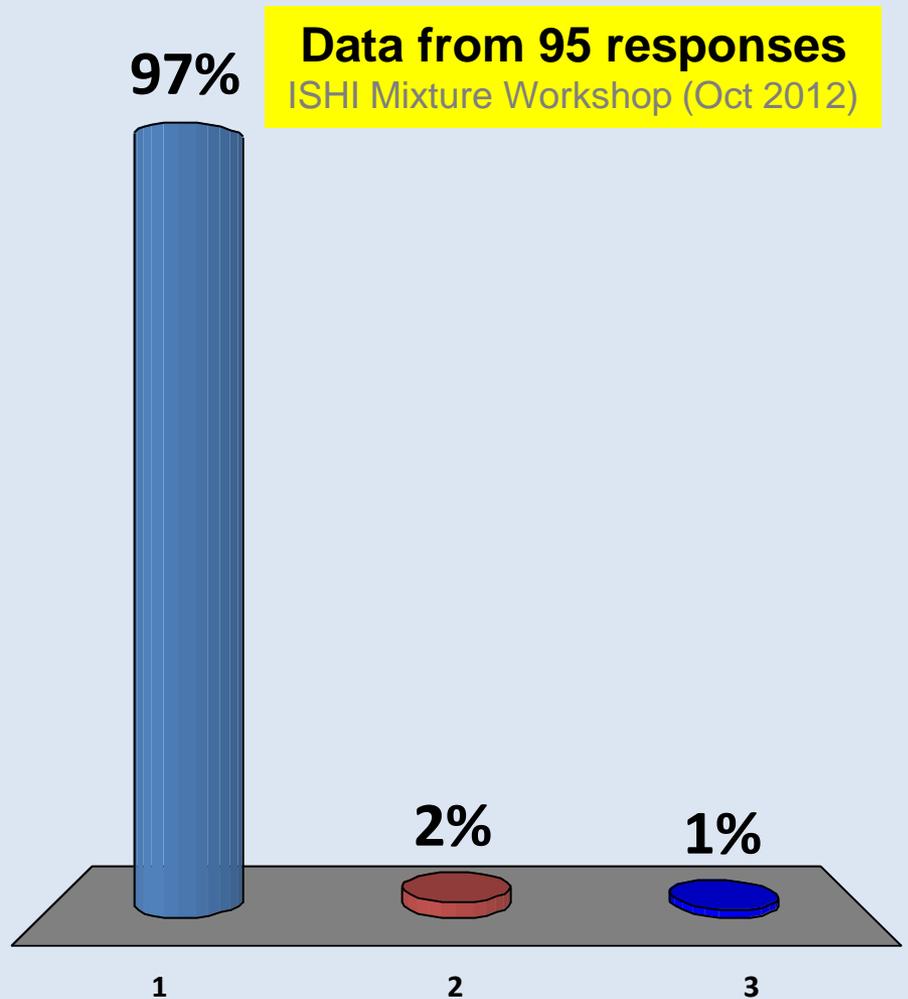
October 15, 2012

Nashville, TN



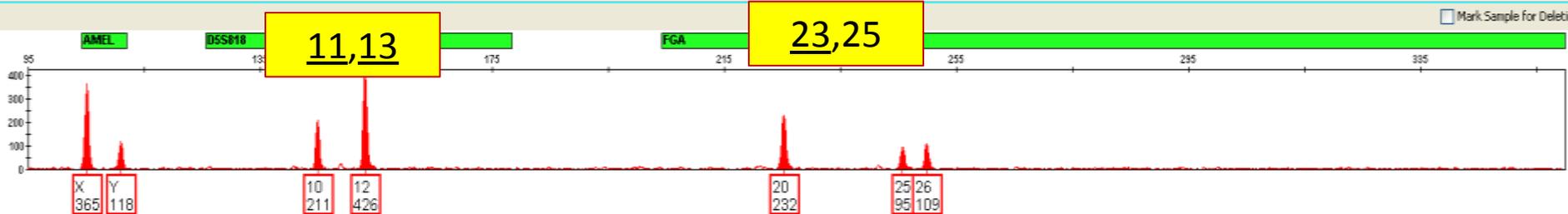
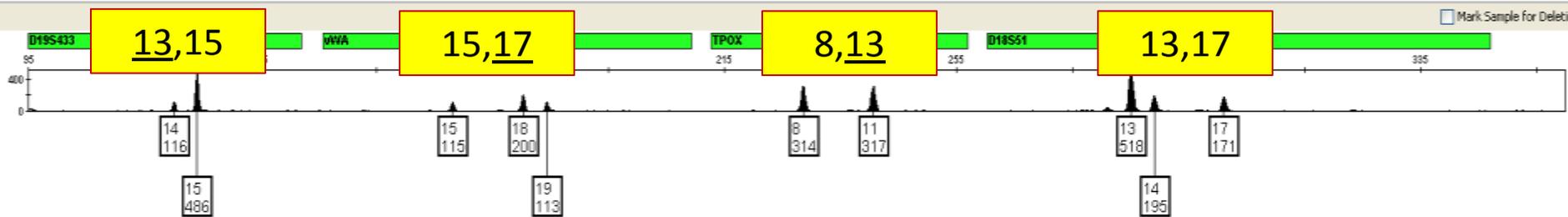
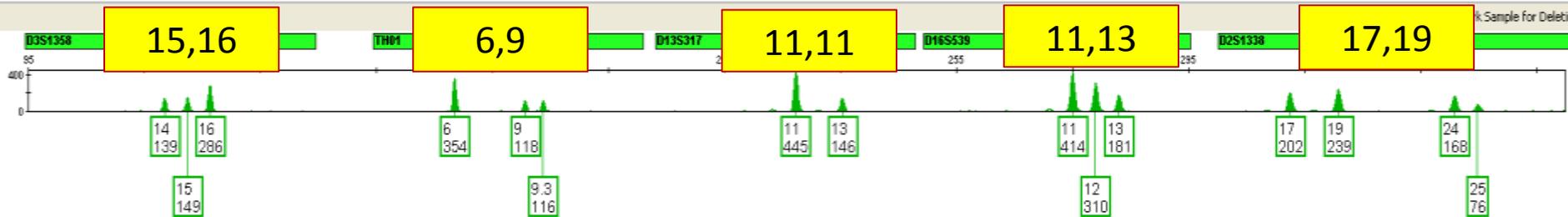
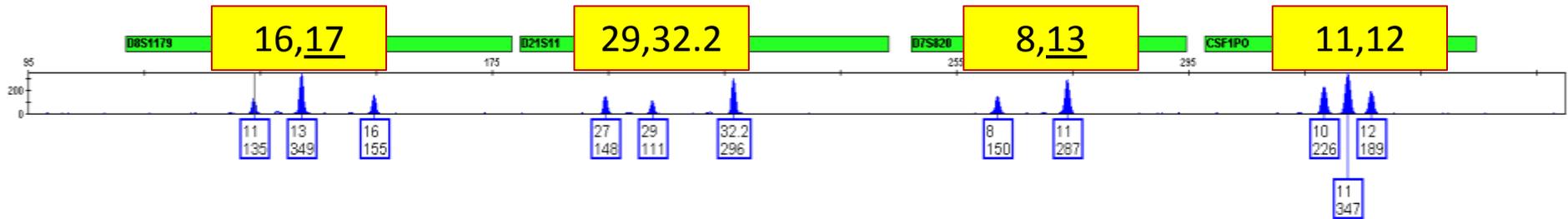
Do your reports contain a 'concluding statement' (i.e. included, excluded)

1. Yes
2. No
3. Don't write reports



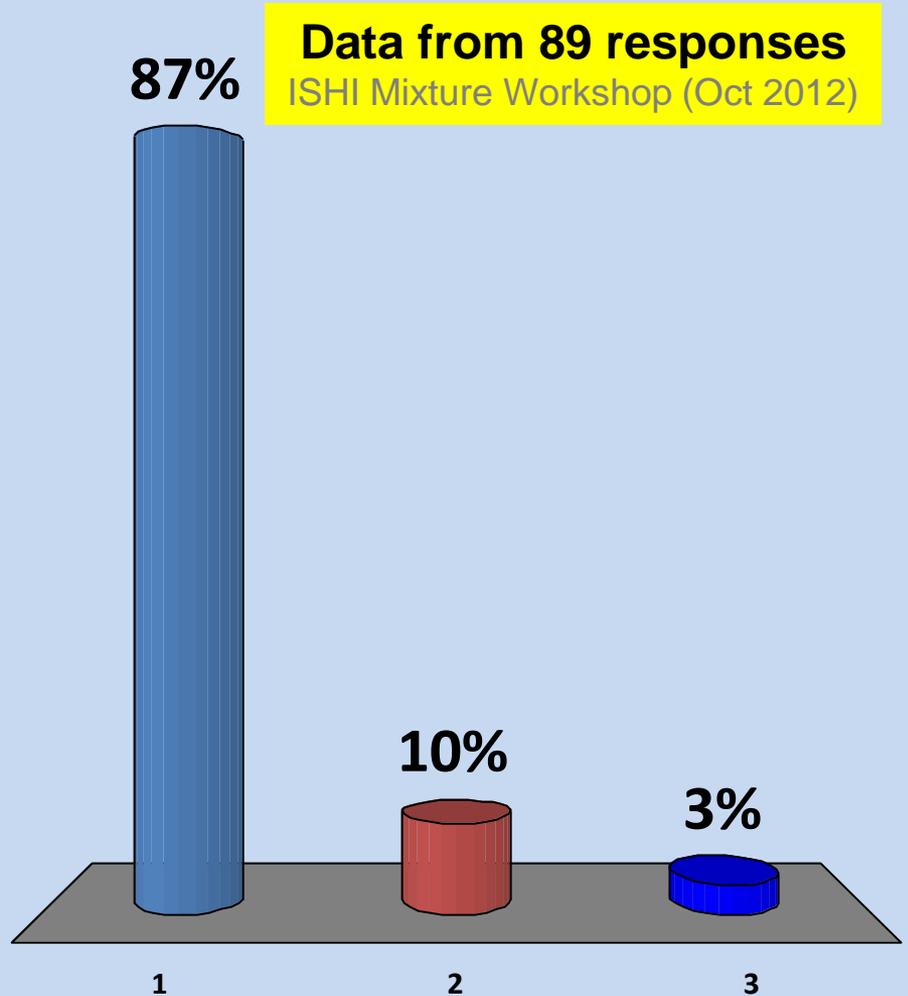
Is this suspect (yellow boxes) not-excluded?

AT	30RFU	
ST	150RFU	
PHR	0.2 (<500RFU)	0.5 (>500RFU)
Major:Minor	4:1	



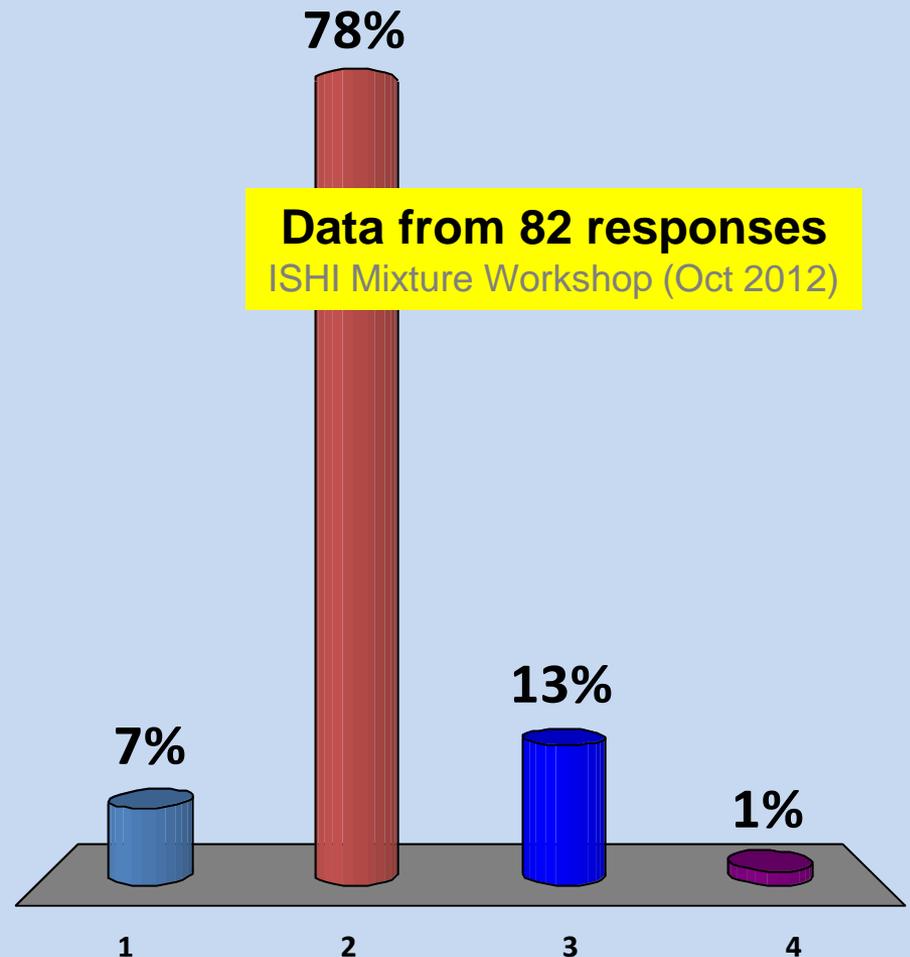
Can the mixture shown in the previous slide be used for exclusion purposes?

1. Yes
2. No
3. I don't know



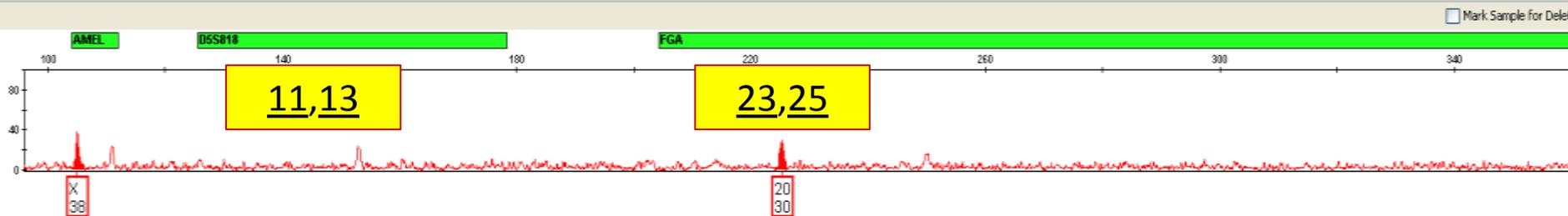
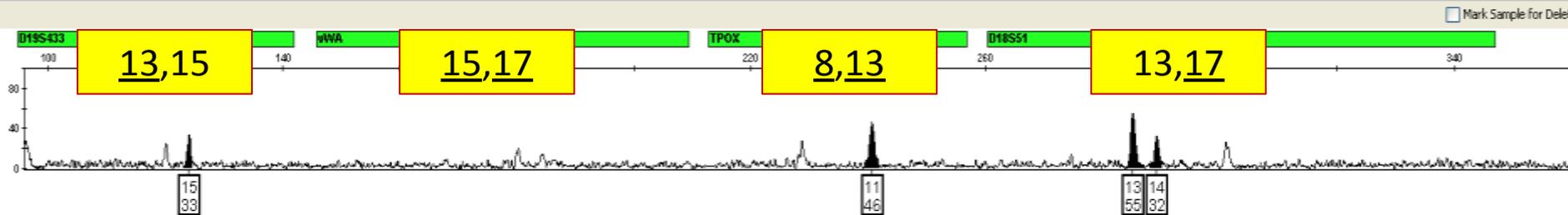
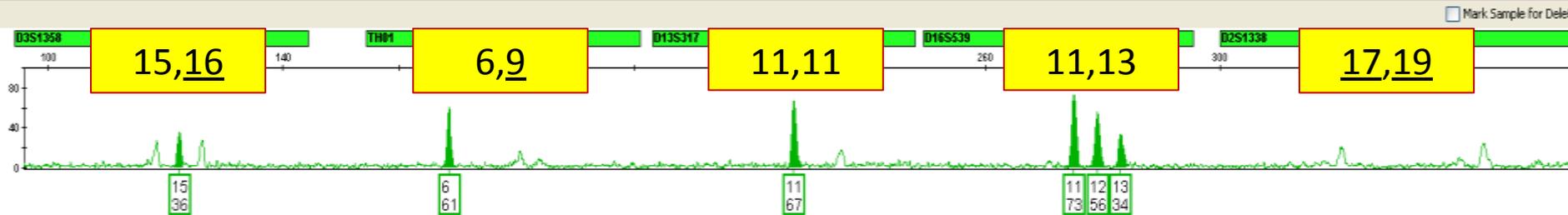
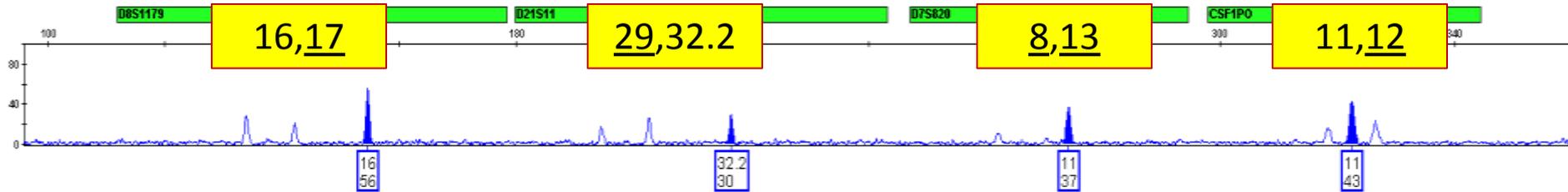
Is the suspect (yellow boxes) included or excluded as a potential contributor to the mixture presented above?

1. Included
2. Excluded
3. Inconclusive
4. The mixture was uninterpretable



Is this suspect (yellow boxes) not-excluded?

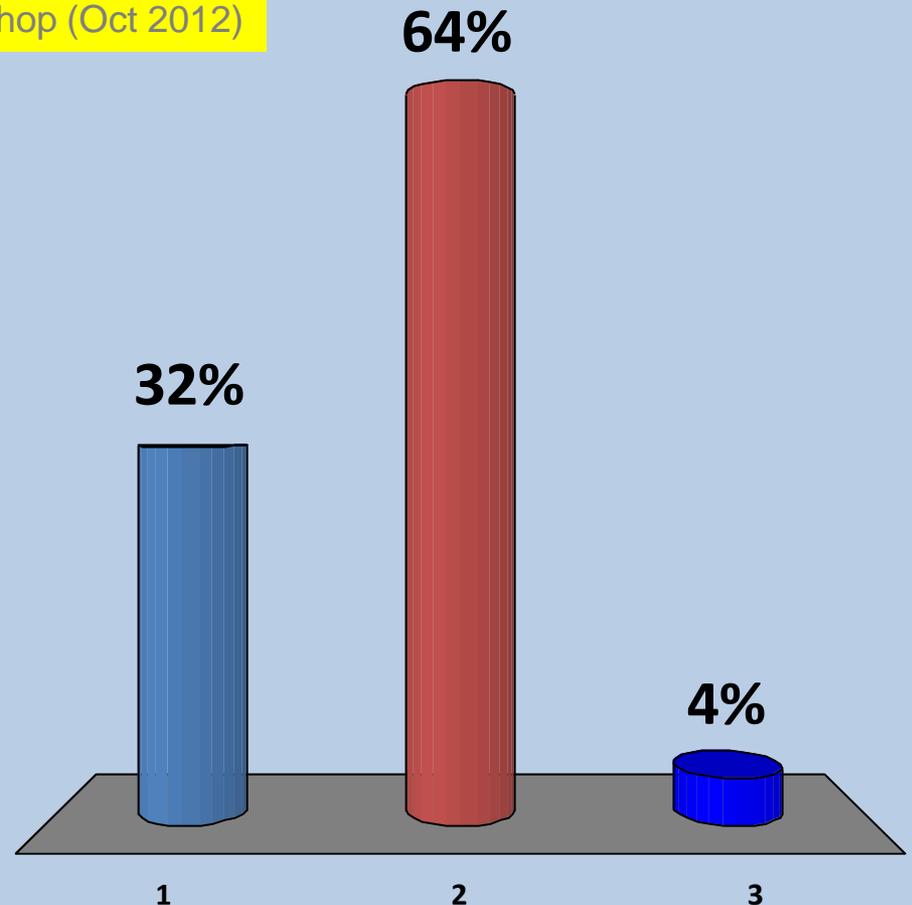
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Can the mixture shown in the previous slide be used for exclusion purposes?

Data from 96 responses
ISHI Mixture Workshop (Oct 2012)

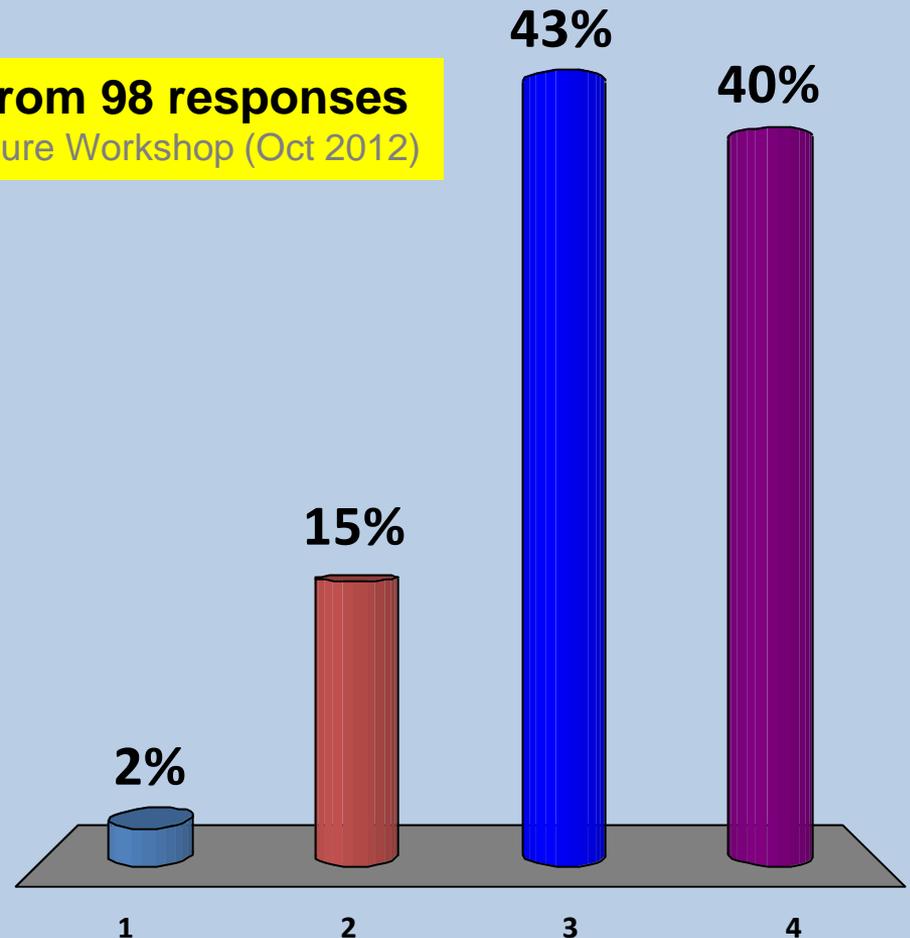
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2. No
3. I don't know



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Data from 98 responses
ISHI Mixture Workshop (Oct 2012)



Statement of the Problem

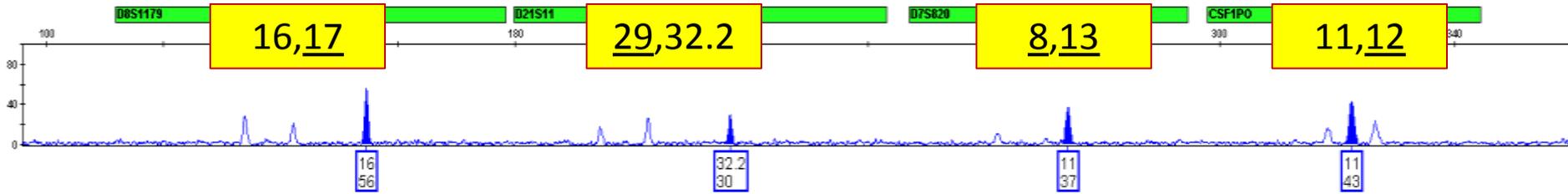
DNA Commission of the International Society of Forensic Genetics: *Recommendations on the Interpretation of Mixtures*

<i>Interpretation Steps</i>	Action
<i>Step 1</i>	Identify the presence of a mixture
<i>Step 2</i>	Designation of contributors
Step "2.5"	<i>Step 3</i> Identify the number of contributors in the mixture
<i>Step 4</i>	Estimation of the mixture proportion or ratio of the individuals contributing to the mixture
<i>Step 5</i>	Consideration of all possible genotype combinations
<i>Step 6</i>	Compare reference samples

Possible to consider "**complexity threshold**" before proceeding?

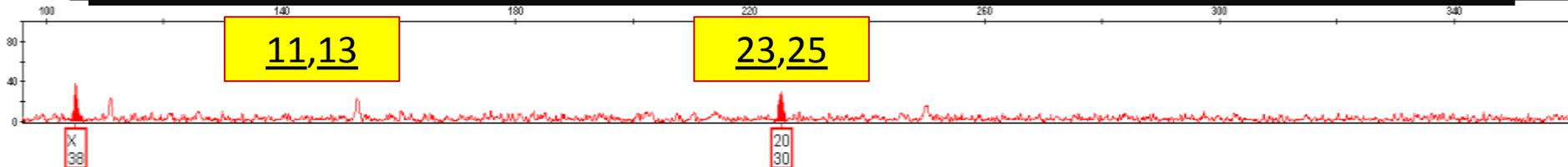
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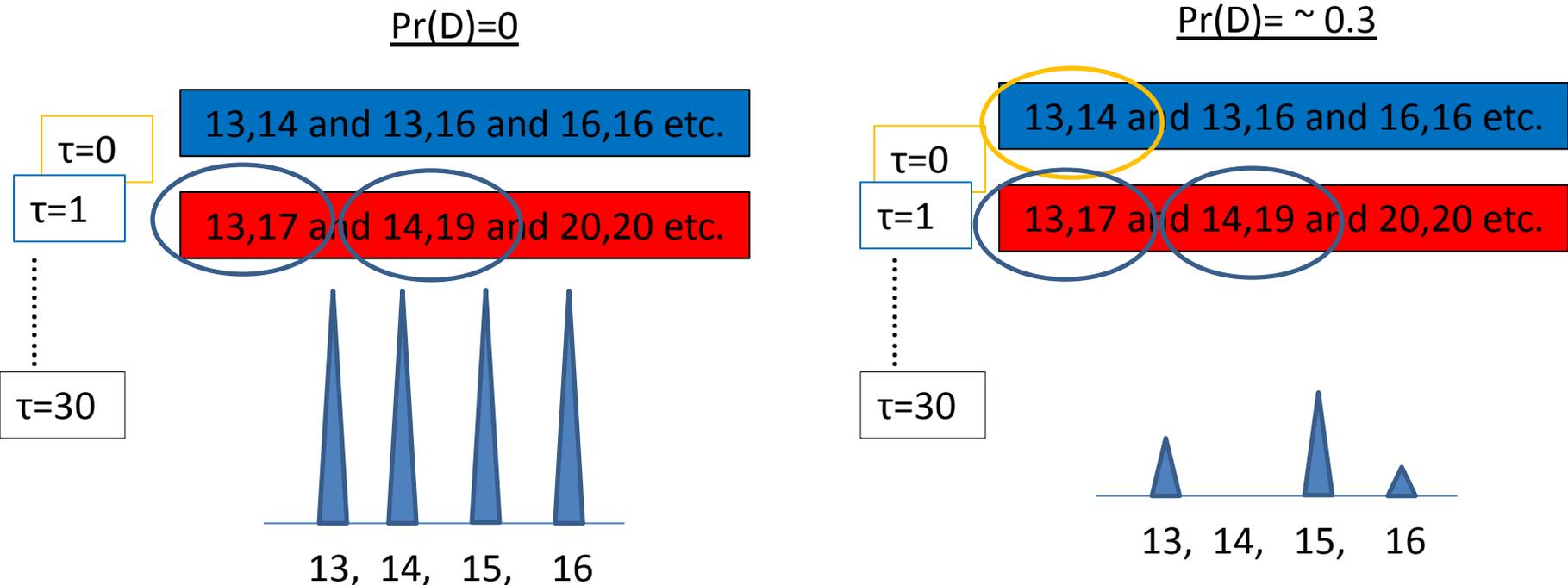
19 discrepancies. Is this enough to exclude this suspect? Or is the mixture missing too much information?

Should we exclude suspect? Or is the comparison inconclusive due to the low-level and complexity?

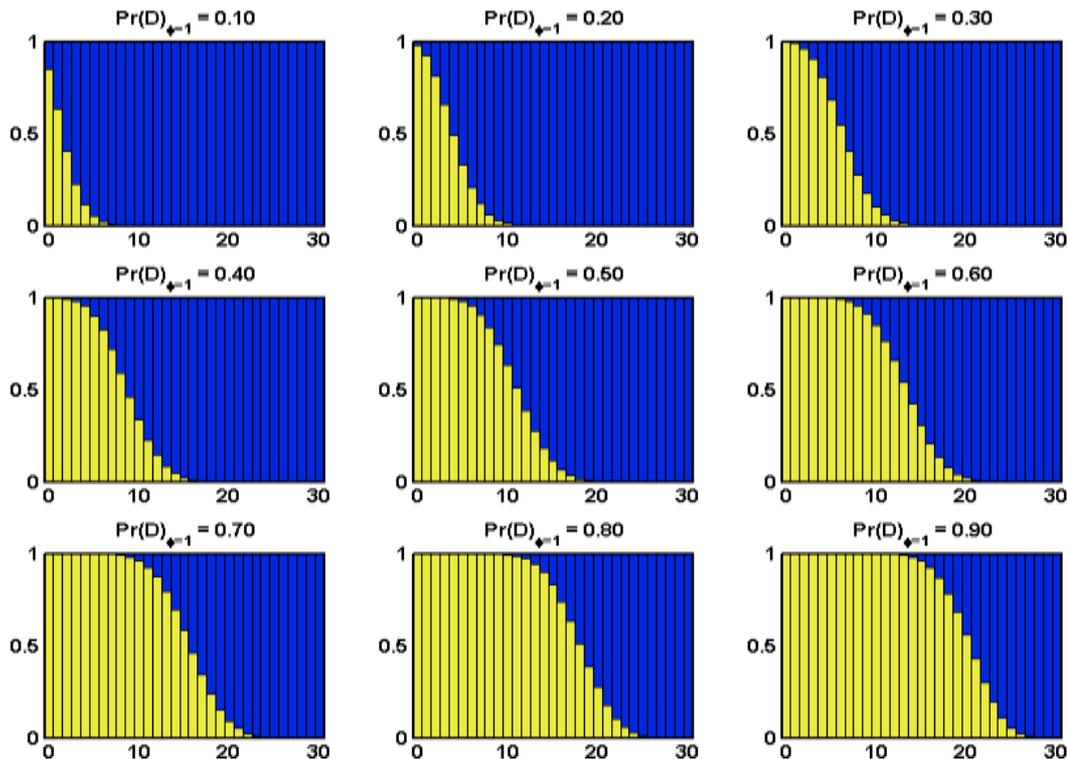
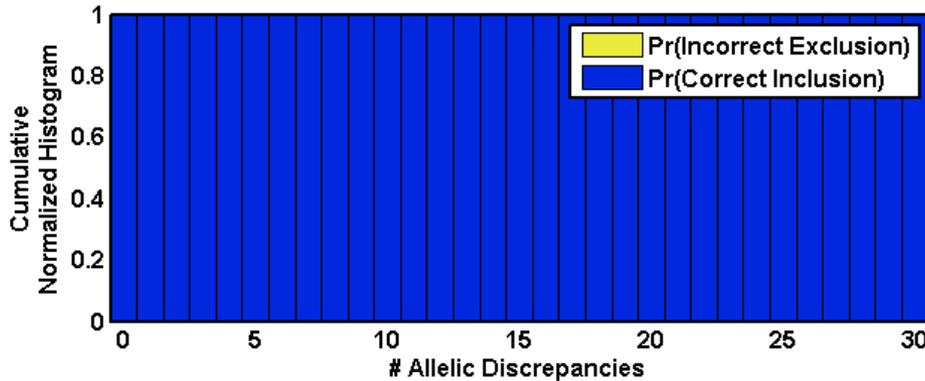


Correct Inclusions vs Exclusions

- Created 10,000 mixtures,
 - 10,000 individuals who ought to have been excluded
 - 10,000 individuals who ought to have been included
 - Perturbing the mixtures with increasing levels of drop-out
 - Determined the proportion of false inclusions and false exclusions with varying levels of “allowed allelic discrepancies” (τ).

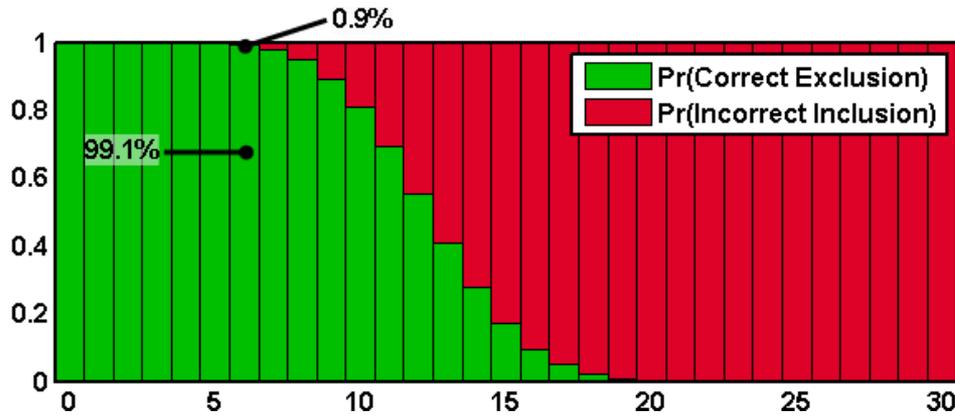


Proportion of Correct Inclusions

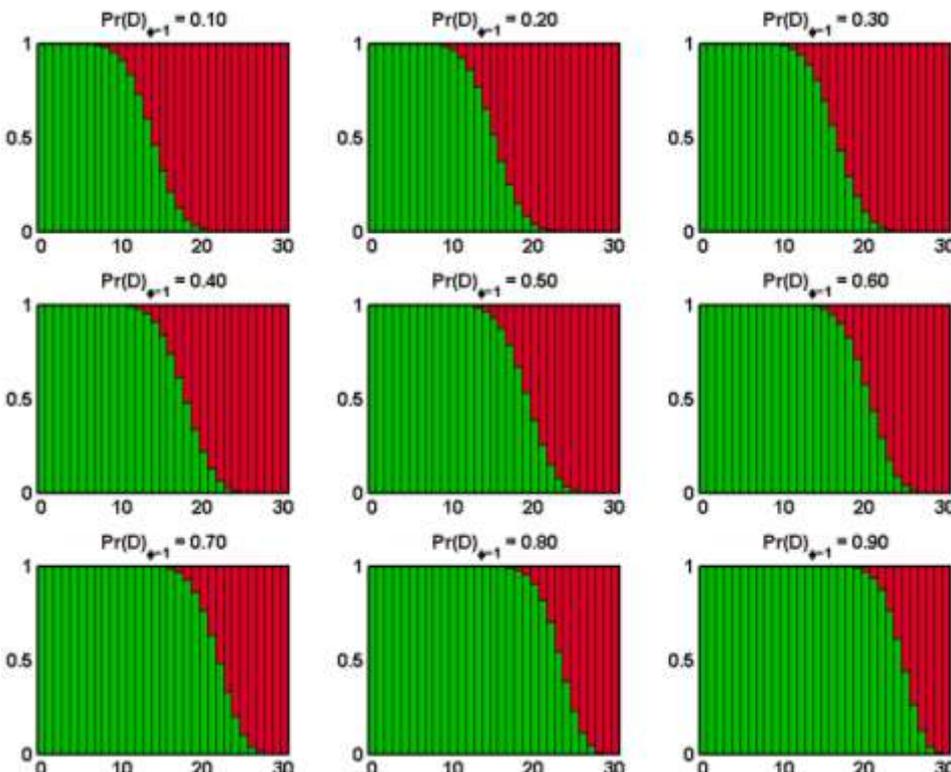


- When $\Pr(D) = 0$, get correct inclusion 100% of the time.
- With increasing levels of DO, **correct inclusion** rates decrease (i.e. you are more likely to exclude a standard who ought to have been included as a potential contributor).
- To alleviate this incorrect exclusion rate, allow for some allelic discrepancy (i.e. allow for some allelic drop-out to explain the inconsistency between standard and mixture).

Proportion of Correct Exclusions



- When $\Pr(D) = 0$, get correct exclusion 100% of the time with < 6 discrepancies
- With increasing levels of DO, **correct exclusion** rates increase (i.e. you are more likely to exclude a standard who ought to have been excluded as a potential contributor)



		Non-Contributor Individual		
		Locus 1	Locus 2	Locus 3
Alleles of Non-Contributor Standard		7, 11	8, 9	13, 15
$\Pr(D) = 0.00$	Detected Mixture Alleles	7, 8, 9, 11	5, 8, 9, 14	11, 13, 14
	Discrepant Allele(s)			15
	Allowed Discrepancy τ_0	Excluded since $\delta=1 > \tau_0$		
	Allowed Discrepancy τ_1	Included since $\delta=1 \leq \tau_1$		
$\Pr(D) = 0.20$	Detected Mixture Alleles	7, 8, 9	8, 9, 14	11, 13, 14
	Discrepant Allele(s)	11		15
	Allowed Discrepancy τ_0	Excluded since $\delta=2 > \tau_0$		
	Allowed Discrepancy τ_1	Excluded since $\delta=2 > \tau_1$		
$\Pr(D) = 0.50$	Detected Mixture Alleles	8	9, 14	11, 14
	Discrepant Allele(s)	7, 11	8	13, 15
	Allowed Discrepancy τ_0	Excluded since $\delta=5 > \tau_0$		
	Allowed Discrepancy τ_1	Excluded since $\delta=5 > \tau_1$		
	Allowed Discrepancy τ_2	Excluded since $\delta=5 > \tau_2$		

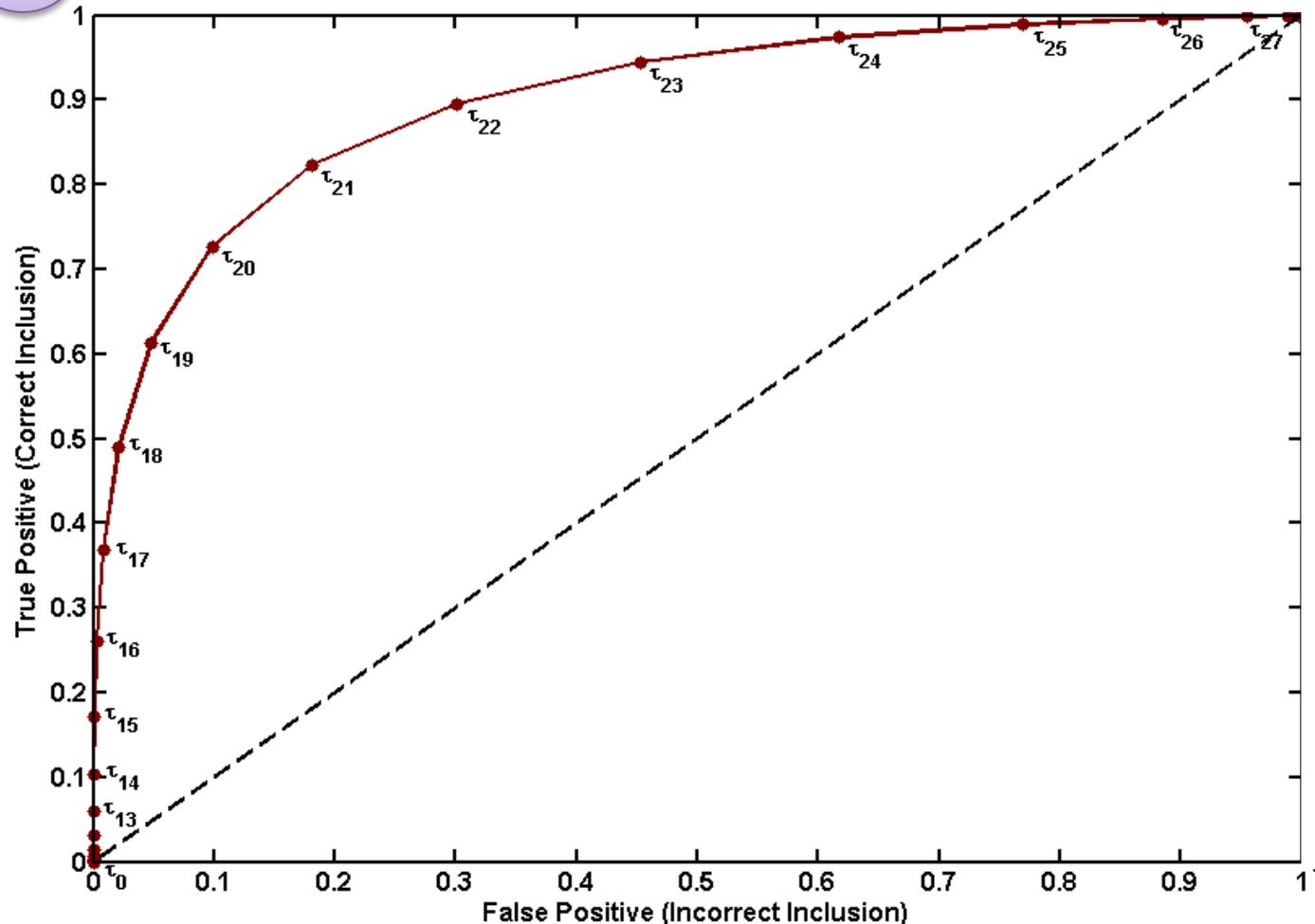
Incorrect Inclusion v Correct Inclusion

– R.O.C. Analysis

- R.O.C, Receiver Operating Characteristic graphs method for visualizing and selecting parameters based on performance rates.
- Two-dimensional charts which plot the true positive versus the false positive rates for a given parameter or classifier.
- If the method is behaving perfectly then the false positive rate is 0 and the true positive rate is 1.

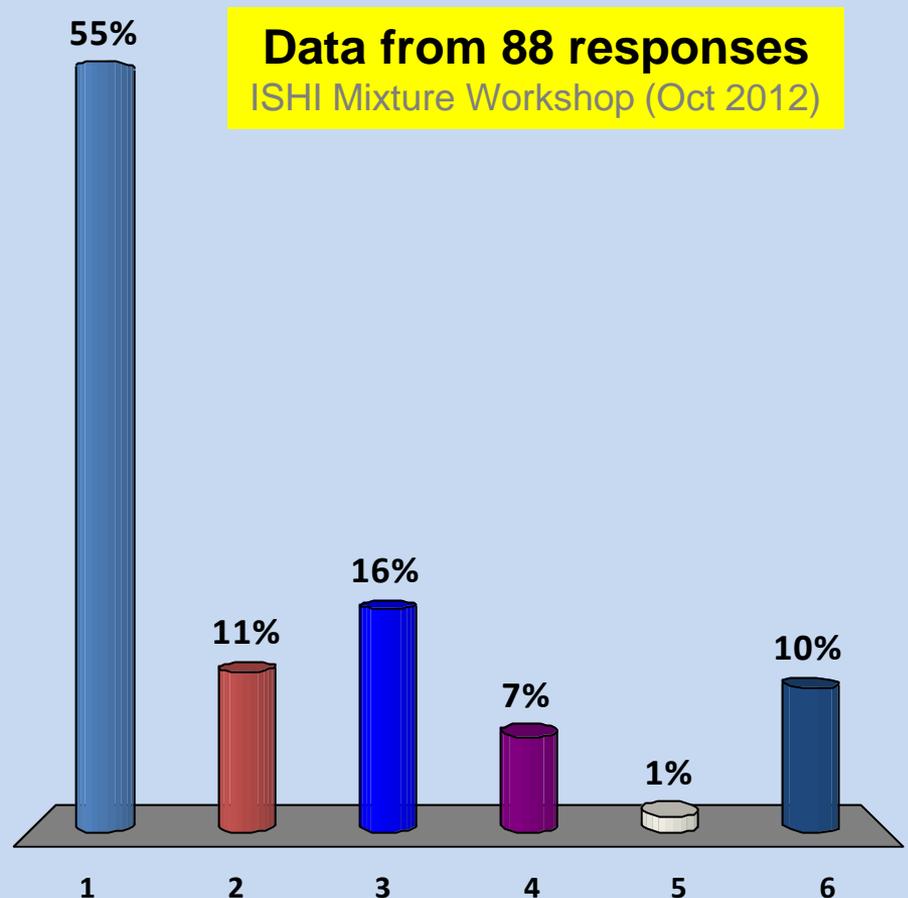


(0,1) = 0 incorrect inclusions and 100% correct inclusions



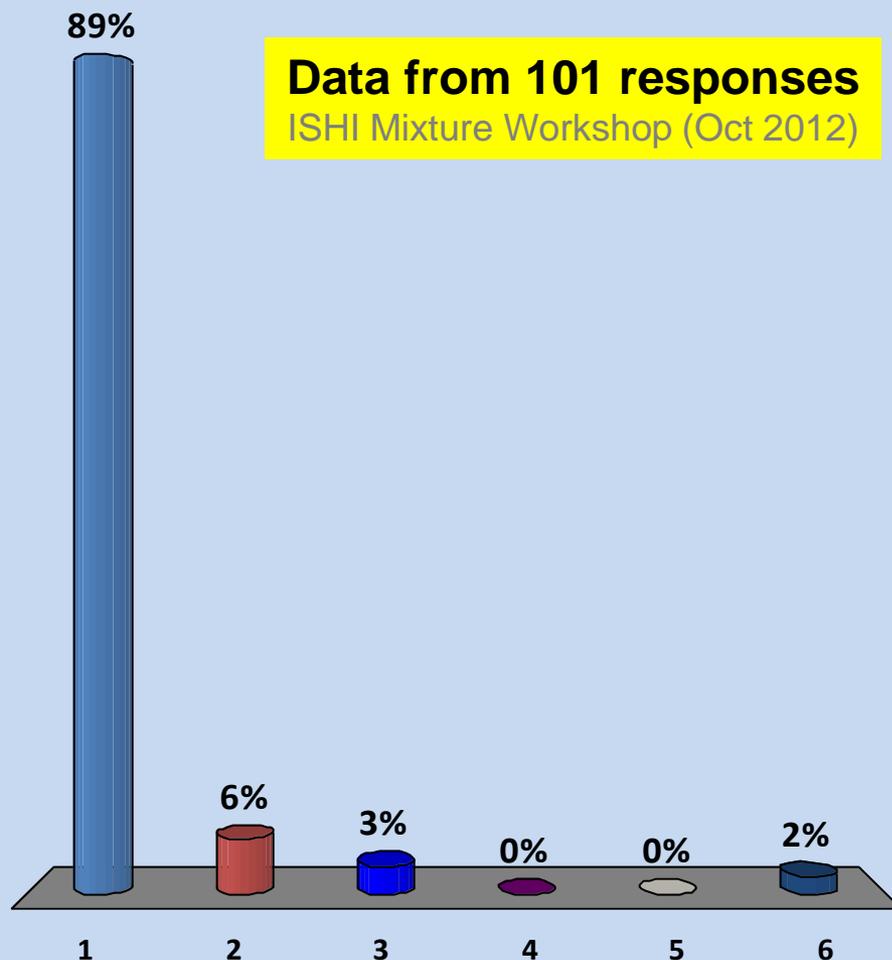
What percentage of the time would you be willing to falsely exclude a standard who in truth should be included?

1. 0%
2. 1%
3. 5%
4. 10%
5. 50%
6. 99%



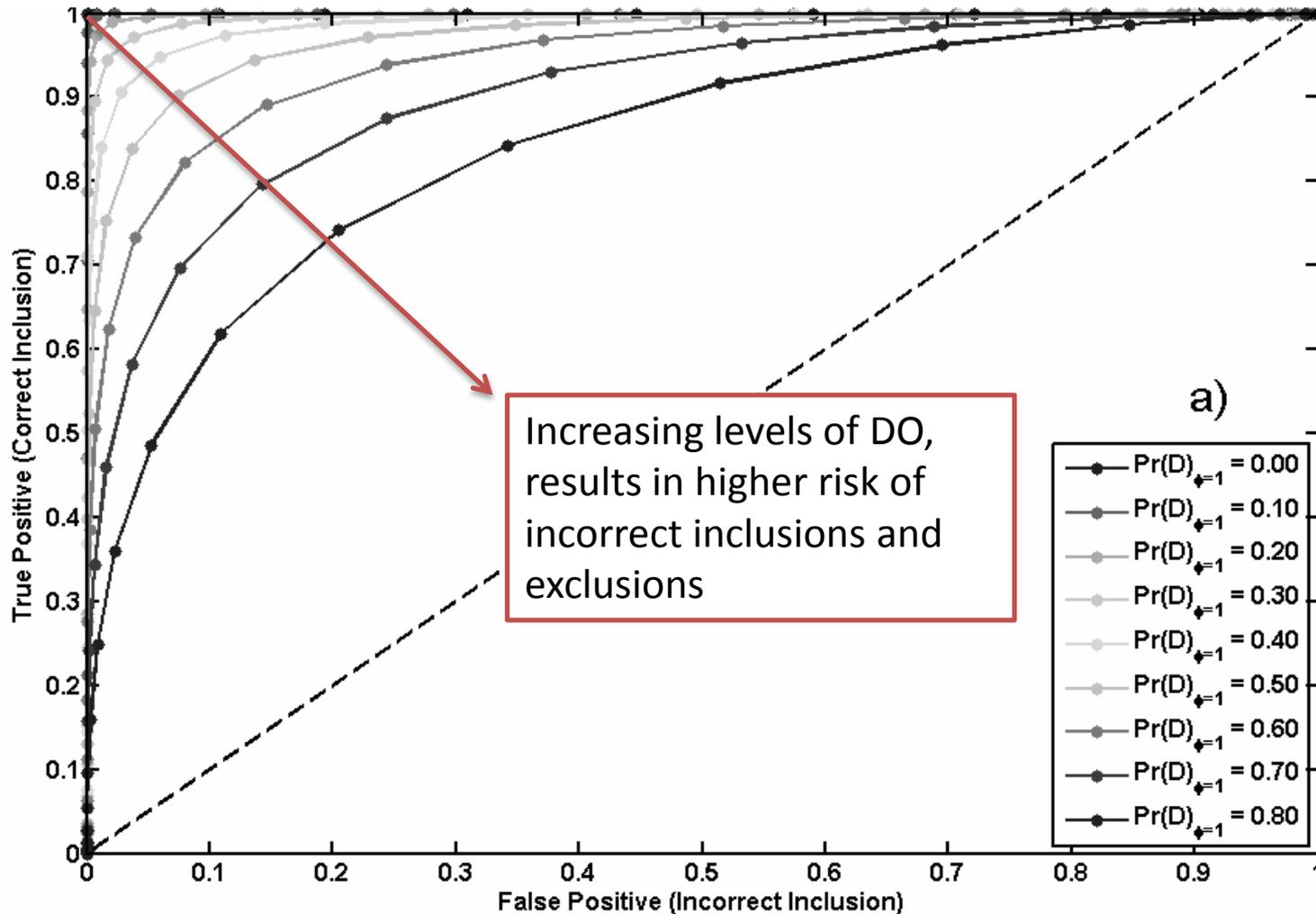
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6. 99%



Incorrect Inclusion v. Correct Inclusion

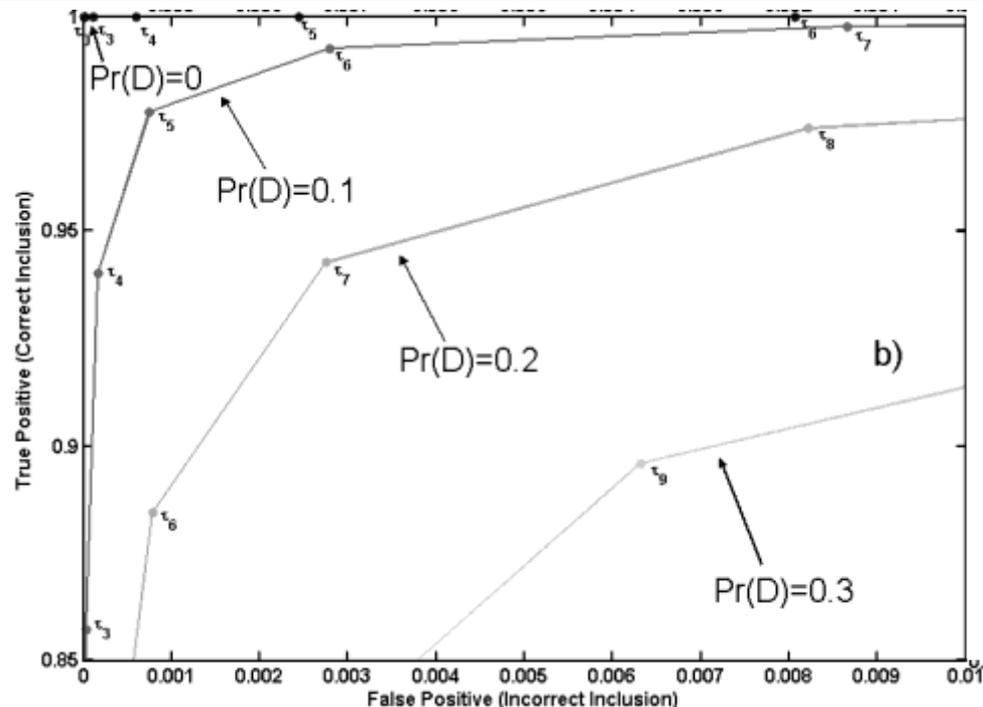
– R.O.C. Analysis



Complexity Threshold, Exclusion Criteria

- If the complexity criteria was set such that the lab does not want a correct inclusion rate < 85% and an incorrect inclusion rate > 1%, then mixtures with suspected $\Pr(D) > 0.3$ should not be interpreted.

Complexity Criteria: Correct Inclusion must be > x%	Complexity Criteria: Incorrect Inclusion must be < y%	Then suspected $\Pr(D)$ must be < z
85%	1%	0.3
95%	0.1%	0.1
100%	0%	0

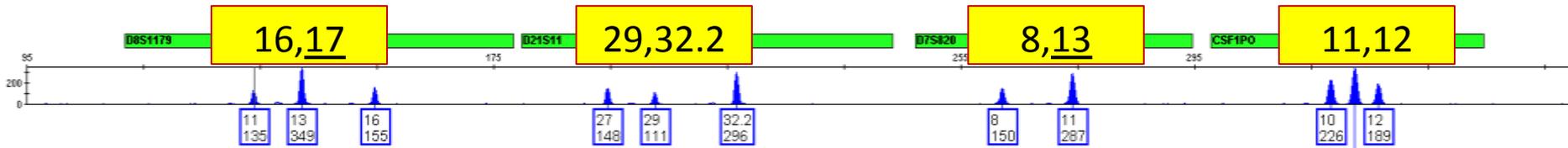


- In our lab, at AT of 30 RFU, $\Pr(D) \sim 0.3$ when $\bar{H} \sim 70$ RFU/allele or ~ 0.1 ng
- $\Pr(D) \sim 0.1$ when $\bar{H} \sim 200$ RFU or ~ 0.2 ng
- $\Pr(D) = 0$ when $\bar{H} > 200$ RFU or > 0.2 ng

If the Complexity Criteria is	And suspected $\Pr(D) =$	Then Exclusion Criteria (allelic discrepancies)	Overall Exclusion Criteria
> 85% correct inclusion and < 1% incorrect inclusion	0.3	10	7
	0.2	9	
	0.1	8	
	0	7	

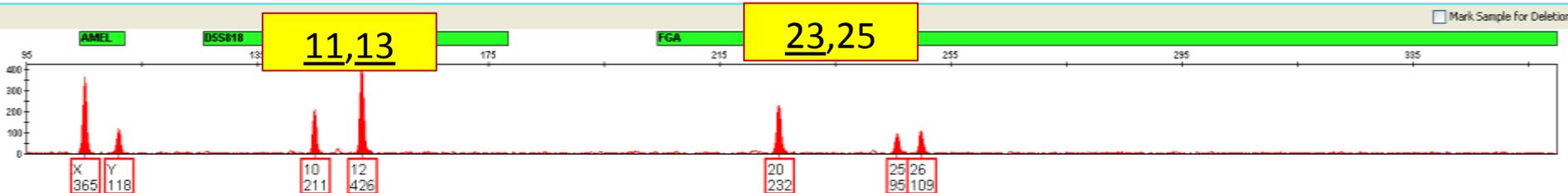
Is this suspect (yellow boxes) not-excluded?

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Complexity Threshold	95% and 0.1% $\bar{H} < 200$ RFU, cannot use for comparison	
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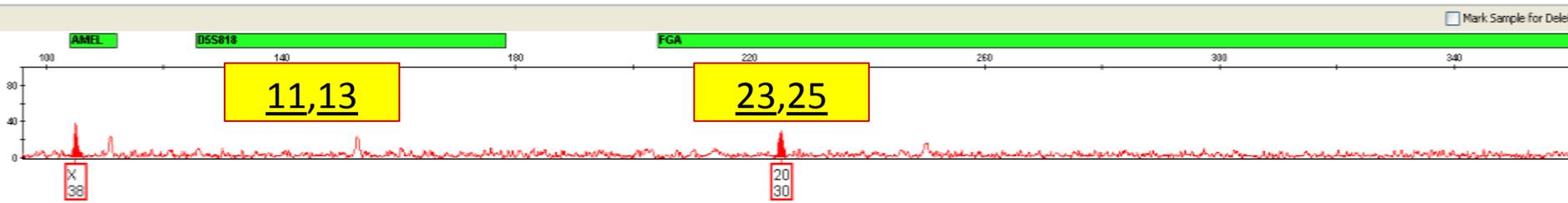
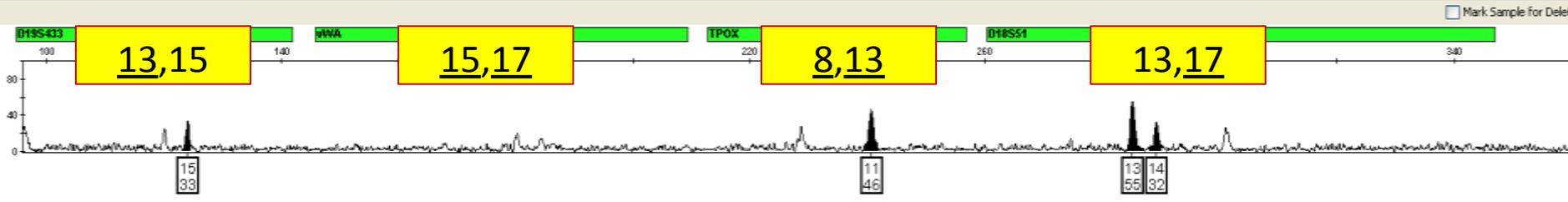
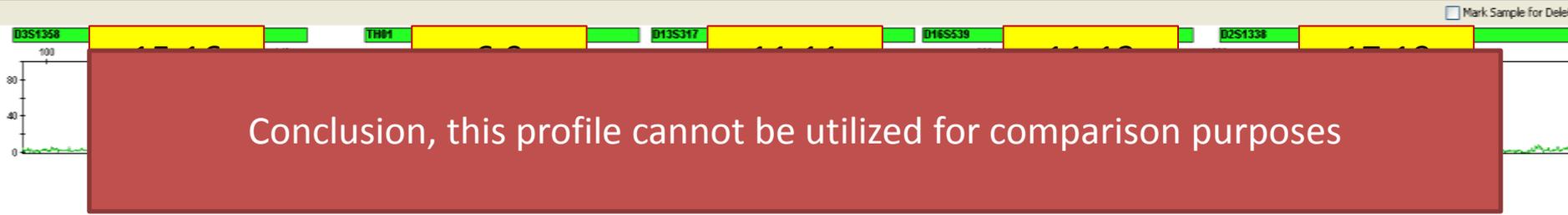
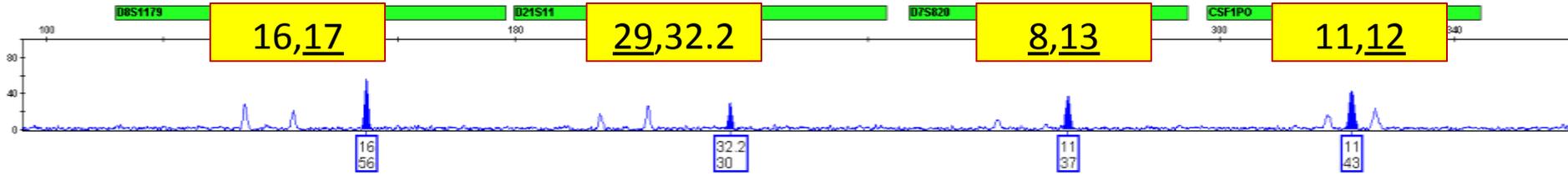
Conclusion, this sample cannot be used for comparison purposes at a complexity threshold of 95% and 0.1%

A more lax complexity criterion such as 85% and 1%, would result in non-exclusion ($\tau=8$) of the standard as a potential contributor.
(Non-exclusion = included OR inconclusive)



Is this suspect (yellow boxes) not-excluded?

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ST	150RFU	
Complexity Threshold	95% and 0.1% \bar{H} < 200 RFU, cannot use for comparison	
PHR	0.2 (<500RFU)	0.5 (>500RFU)
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Conclusions

- Inherent risk of false inclusions and exclusions when using mixed, low-level samples for comparison purposes
- The level of drop-out, hence peak height, can be used to aid in determining whether the profile is suitable for comparison purposes
- This decision can be made BEFORE comparison to knowns
- R.O.C. analysis can be used as a tool to determine complexity and exclusion criteria
- If there is a need to “explain” why there are > 8 allelic discrepancies while still not excluding the standard, then with 2-person mixtures, there is a > 1% chance you are including a known that would have been excluded had you had a sufficient quantity of DNA and more stringent complexity guidelines.